

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Atsuhiko OGURA, et al.

Serial No. 10/810,305

Examiner: Kiliman, Leszek B.

Filed: March 26, 2004

Group Art Unit: 1773

For: POLYETHYLENE GLYCOL-MODIFIED SEMICONDUCTOR
NANOPARTICLES, METHOD FOR PRODUCING THE SAME,
AND MATERIAL FOR BIOLOGICAL DIAGNOSIS

RULE 132 DECLARATION

I, Atsuhiko OGURA, a Japanese citizen residing at 3-1-14-103, Tanaka,
Tsuchiura-shi, Ibaraki 300-0048 Japan, declares as follows:

1. I am a co-inventor of the above-identified patent application.
2. In March 1990, I graduated from Tohoku University, where I was enrolled at Faculty of Engineering, Department of Applied Chemistry. In April 1990, I was employed by NOF Corporation, and was attached to Chemical & Explosives Division, where my main work was about research and development of propellants. In November 1996, I was attached to Tsukuba Research Laboratory of the same company, where my main work was about research and development of block copolymers for drug delivery system until March 2000. From April 2000 to March 2003, my main work was about research and development of PEGylated quantum dots for biological diagnosis in the same Laboratory, as well as in Japan Science and Technology Agency. Since April 2003, I have been involved in researches in the same Laboratory, where my main work is about researches and development of functional monomers.
3. I am a co-author of "Preparation of Water-soluble PEGylated Semiconductor Nanocrystals", Chemistry Letters, Vol. 33, No. 7 (2004), pp840-841.
4. I am familiar with the specification and the claims of this application.
5. I have conducted the following experiments under my direct supervision.

EXPERIMENTS

Experiment 1

Preparation of PEG-modified CdSe-ZnS Semiconductor Particles

A CdSe-ZnS solution containing 10 $\mu\text{mol/ml}$ CdSe was prepared in accordance with Preparation Example 1 of the present specification. On the other hand, hetero-bifunctional PEG was prepared in accordance with Preparation Example 3 of the present specification, except that the number average molecular weight of PEG was adjusted to 400, 1000, 2000, 5000, 7000, and 10000.

To 100 μl of the CdSe-ZnS solution, 10 μmol of PEG and 1 ml of phosphate buffer mixed with no salt, 9 μmol of CdCl_2 , or 9 μmol of ZnCl_2 were added, and vigorously stirred in oblique rays at a room temperature. After 30 minutes, 10 ml of n-hexane and 9 ml of phosphate buffer were added, and vigorously stirred for additional 5 minutes. The mixture was then left still to phase-separate. Under UV irradiation (254 nm), fluorescence in each phase was observed. The results are shown in Table 1.

It was demonstrated that, when no salt was added, or a salt of Zn was used, which belongs to the same sub-group IIB of the Periodic Table as Cd, PEGs of only limited molecular weights could be bonded to the surface of CdSe-ZnS. On the other hand, when CdCl_2 was used, even PEGs of higher molecular weights could easily be bonded to the surface of CdSe-ZnS.

Table 1

Additive	Number average molecular weight of hetero-bifunctional PEG (Sample #)					
	400	1000	2000	5000	7000	10000
None	○(1)	×	×	×	×	×
CdCl_2	○(2)	○(4)	○(5)	○(6)	○(7)	△
ZnCl_2	○(3)	△	×	×	×	×

- Fluorescence was observed only in the lower (aqueous) phase
- △ Fluorescence was observed in both upper and lower phases
- ×

Fluorescence was observed only in the upper (organic) phase

Experiment 2

Evaluation of Stability of PEG-modified CdSe-ZnS Semiconductor Particles

The seven samples (1) to (7) above, which had been successfully pegylated, were subjected to a test for stability under the following conditions. The results are shown in Fig. 1.

Test samples: PEGylated CdSe-ZnS in phosphate buffer (5 ml) + BSA
(bovine serum albumin) in phosphate buffer (100 µg/ml) (15 ml)

Test temperature: 37 °C (in incubator)

Sampling Time: at 3, 7, 24, and 48 hours

Analysis: with spectrophotometer

Wavelength: 700 nm

Analysis Temperature: 37 °C (under stirring)

The transmittance of Samples (1), (2), and (3), all with PEG 400, was declined with time. This is because the semiconductor particles were coagulated due to non-specific physical adsorption, which lead to reduced transmittance. On the other hand, the transmittance of Samples (4) to (7), with PEGs of higher molecular weights, was hardly declined, which means that high dispersion stability was maintained.

From the above results, it is understood that, by binding hetero-bifunctional PEG having a number average molecular weight of 1,000 to 7,000 to the surface of CdSe-ZnS in the presence of a cadmium salt, the resulting product exhibits high dispersion stability and inhibitory property on non-specific adsorption. This product can make it possible to detect trace substances highly sensitively in biological diagnosis.

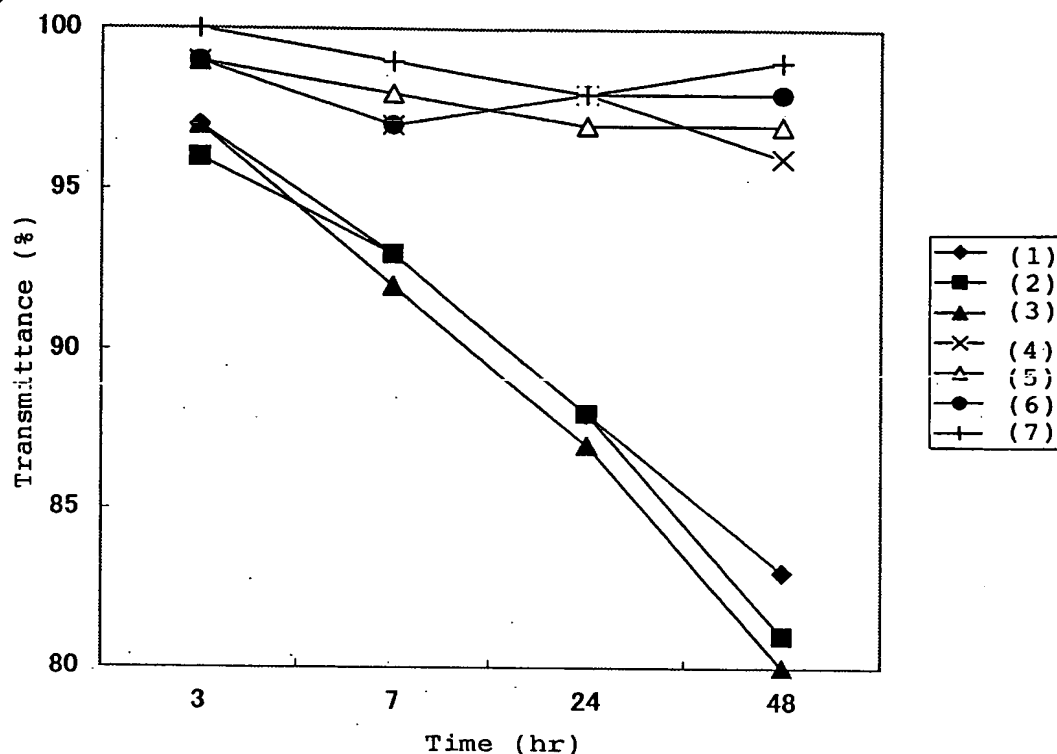
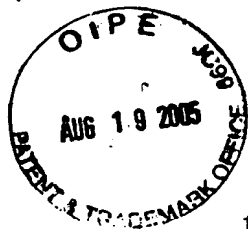


Fig. 1 - Results of Test for Dispersion Stability of PEGylated CdSe-ZnS

6. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United State Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Declared and signed at Tsukuba, Japan
Dated this 11 day of August, 2005

Atsuhiko OGURA

Atsuhiko OGURA